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PRE-APPEAL BRIEF REQUEST FOR REVIEW

Docket Number (Optional): AM100039

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on May 30, 2007

Signature: Carol E. Rozek

Typed or printed name: Carol E. Rozek

Application Number	Filed
10/019,163	12-20-01

First Named Inventor: Lakhotia, Sanjay

Art Unit	Examiner
1645	Ford, Vanessa L.

Applicant requests review of the final rejection in the above-identified application. No amendments are being filed with this request.

This request is being filed with a notice of appeal.

The review is requested for the reason(s) stated on the attached sheet(s).
Note: No more than five (5) pages may be provided.

I am the

- [ ] applicant/inventor.
- [ ] assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)
- [X] attorney or agent of record. Registration number 36,993
- [ ] attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34 _____

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May 30, 2007
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

- [ ] *Total of _____ forms are submitted.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/019,163	Confirmation: 1674
Applicant	:	Lakhotia and Biehl	
Filed:	:	December 20, 2001	
For	:	Extraction of Integral Membrane Proteins	
Art Unit	:	1645	
Examiner	:	Vanessa L. Ford	
Docket No.	:	AM100039	
Customer No.	:	25291	

May 30, 2007

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRE-APPEAL BRIEF REQUEST FOR REVIEW

Sir:

In the above-referenced application, an Office Action dated September 18, 2006 rejected claims 1-16 under 35 U.S.C. § 103(a) as allegedly being obvious over cited prior art. A Final Office Action dated January 25, 2007 maintained the rejection of claims 1-16 under 35 U.S.C. § 103(a) in view of the same art. The basis for maintaining the rejection was the same as that provided in the Office Action dated September 18, 2006. As such, the claims in the present application have been twice rejected and qualify for appeal. Accordingly, the following remarks are being submitted together with a Notice of Appeal under 37 C.F.R. § 41.31 in support of a Pre-Appeal Brief Request for Review.

Applicants believe that the outstanding rejection of record is improper and without basis. In support of this position, Applicants present the following legal and/or factual deficiencies in the rejection.

Pending Claims

The pending claims are drawn to a process of differential detergent tangential flow diafiltration for extracting native or recombinantly-expressed gram-negative integral (inner and outer) membrane proteins from bacteria or bacterial host cells containing a

recombinant vector. This process has several advantages over alternate processes. First, this process combines the clarification and extraction processes into one unit operation. The product is extracted from the cells and it is separated from cell debris with only one continuous diafiltration process. The second advantage is that the membrane proteins are extracted in a semi-purified state, which simplifies the downstream processing steps. Finally, this process **avoids the use of centrifugation** and is therefore more amenable to scale-up.

Prior to the commencement of the extraction process of this invention, an integral membrane protein from a gram-negative bacterium is expressed in a homologous or heterologous bacterial host cell by conventional methods, or the native bacterium is isolated. The fermentation broth is then **lysed** by passing it through a homogenizer to commence the extraction process.

The **lysed fermentation broth** is then subjected to a differential detergent extraction process utilizing tangential flow filtration (TFF) technology. In this process the **lysed cells** are diafiltered with a specific sequence of buffer solutions using a tangential flow system that includes a permeable membrane with a defined size cut-off or opening. The sequence of buffer solutions is chosen to solubilize inner membrane proteins first and then to solubilize the outer membrane proteins. During diafiltration, the solubilized proteins of approximate size less than the molecular weight cut-off of the membrane pass through with the permeate, while larger molecules and unsolubilized proteins are retained.

The buffer solutions are then changed and a detergent is introduced to solubilize and extract outer membrane proteins. The sequence of buffer and detergent steps is controlled to extract the desired outer membrane protein in a selective manner. The extracted protein is then purified by conventional means such as ion exchange chromatography.

Rejection on Appeal

At page 2 of the Final Office Action dated January 25, 2007, claims 1-16 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious and unpatentable over Green et al. (U.S. Patent No. 5,780,601) in view of Nicholson (U.S. Patent No. 5,681,936). The Examiner contends that one skilled in the art would have been motivated to combine the tangential flow filtration (TFF) taught by Nicholson with the

differential detergent extraction taught by Green et al. to extract bacterial proteins because Nicholson teaches that using TFF has the benefit of producing high yield proteins that are entirely homodimeric.

Basis for Request for Pre-Appeal Review

Applicants respectfully submit that the Examiner has failed to articulate why it would have been apparent to one of ordinary skill in the art to combine the prior art elements in the way the claimed new invention does. *KSR International Co. v. Teleflex Inc.*, 550 U.S. __, [82 USPQ2d 1385, 1396] (2007). Instead, the Examiner has read into the prior art the teachings of the claimed invention to formulate her rejection.

The Examiner states that Nicholson is used to teach that after the cells have been lysed, TFF is used to remove low molecular weight components (Final Office Action, p. 5), when in fact, Nicholson makes no mention whatsoever of lysing the cells and using TFF on lysed cells. Rather, Nicholson uses TFF after cellular debris has been removed from the cell culture medium by centrifugation and filtration (see Example 3 and claim 1). Thus, the Examiner has improperly relied on only the TFF component of Nicholson to support the rejection, rather than look at what the entire reference teaches. References must be taken in their entireties, including those portions that argue against obviousness. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 203 USPQ 416, 420 (Fed. Cir. 1986). It is impermissible within the framework of section 103 to pick and choose from a reference only so much of it as will support a conclusion of obviousness to the exclusion of other parts necessary to a full appreciation of what the reference fairly suggests to one skilled in the art. *Id.* at 419.

Specifically, Green et al. disclose a method of purifying P4 by differential detergent extraction utilizing methods such as differential sedimentation (col. 4), gradient sedimentation (col. 4), and centrifugation (col. 14), none of which rely on molecular size differences. The only requirement of these processes is that the membrane proteins be either soluble or insoluble; these proteins are not required to pass through a diafiltration membrane. Green et al. do not teach TFF and combining Green et al. with Nicholson does not remedy this deficiency.

Nicholson discloses a method for recovering and purifying human interleukin-5 (IL-5) from a cell culture medium having other (contaminating) proteins in a single chromatographic step. Nicholson's method first requires removing cellular constituents

and debris from the cell culture medium **by centrifugation and/or filtration** (col. 5, lines 15-19; col. 6, line 42; col. 10, lines 33-38; claim 1, step A). Then, low molecular weight components are removed from the cell culture medium by ultrafiltraion, diafiltration or dialysis (col. 5, lines 19-23; col. 10, lines 50-65; claim 1, step B). Next, the conditioned cell culture medium is adjusted to the calculated pI value of mature IL-5 (col. 5, lines 31-35; col. 6, lines 42-44; claim 1, step C). Lastly, the conditioned cell culture medium of the previous step is passed through, in any order, tandem linked anion- and cation-exchange columns (col. 5, lines 1-4; col. 6, lines 44-45; col. 10, lines 66-67 through col. 11, lines 1-4; claim 1, step D).

In the presently claimed invention, the inventors detail the utilization of TFF in a process in which the first step is *lysis* of bacterial cells, combined with differential detergent extraction of membrane proteins. This initial lysis will release nucleic acids and contaminating proteins, situations that clearly do not apply to the purification described by Nicholson. Since one of the main benefits of TFF known at the time was its low sheer forces and ability to keep cells intact, it is unlikely that one skilled in the art would be motivated by Nicholson to try TFF for process purification of the bacterial membrane proteins. Furthermore, one skilled in the art would know that differential detergent extraction is performed at or above the critical micelle concentration (CMC) of the detergent, assuring that the detergent and extracted membrane proteins will mostly be in micelles. The unobviousness of the claimed invention is the ability to use TFF with all of these factors that one would have thought should mitigate its use. Nicholson presents the use of TFF as a method suited to situations where one has a soluble protein **after removal** of the producing cells. Nicholson makes no references to any of the factors that the inventors overcame in the successful use of TFF in the claimed invention, including how to ascertain that one could move detergent micelles through a tangential flow filter without clogging, how to choose filters for such a use, how to change detergents, what criteria are used to judge such parameters, etc. Nicholson at best serves as a mention of TFF as a possible methodology for separating low molecular weight components from high molecular weight ones, but does not prompt those of ordinary skill in the art to use TFF in the situation faced by the inventors of the present invention.

In sum, Nicholson's method specifically allows the cells to remain intact and not be lysed, whereas the claimed invention specifically deals with the critical combination of

lysed bacterial cells and tangential flow filtration. As stated previously (*see* July 6, 2006 Amendment, page 3), TFF is typically used in processes to avoid the lysis of cells, as the lysed content tends to clog filters. Thus, one skilled in the art would be motivated to **avoid** using TFF when dealing with a preparation of lysed bacterial cells. That the inventors of the claimed invention successfully applied TFF to lysed bacterial cells supports the conclusion that the claimed invention would not have been obvious to those skilled in the art. "[W]hen the prior art teaches away from combining certain known elements, discovery of a successful means of combining them is more likely to be nonobvious." *KSR International Co. v. Teleflex Inc.*, 550 U.S. __, [82 USPQ2d 1385, 1395] (2007) *citing United States v. Adams*, 383 U.S. 39, 51-52 (1966).

Conclusion

In view of the foregoing, Applicants submit that claims 1-16 are in condition for allowance, and respectfully request that the instant application pass to issue.

Respectfully submitted,

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